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Factors Affecting the Rate of Flashing and Loss of Luminescence in an Asian Land Snail, *Dyakia striata*

by

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Abstract. The Asian snail *Dyakia striata* is the only terrestrial gastropod known to produce light. Fifteen experiments tested the effects of selected social, environmental, and reproductive factors on its rate of flashing and on the loss of luminescence in some individuals; the factors were age, group size, photoperiod, temperature, diet, starvation, and reproductive maturity. An additional experiment provided data on survival of non-luminescent individuals for comparison with luminescent individuals maintained under identical conditions. Flashing rates were (1) lowest among all experiments for a cool temperature, (2) highest among three diets for a highly proteinaceous vegetable, (3) higher for grouped than isolated snails, (4) higher for groups of five individuals than groups of 2, 10, or 15, and (5) higher for young than old snails. Loss of ability to produce light did not affect survival. It occurred most often in isolated snails under extremes of photoperiod (*i.e.,* continuous light) and temperature (*i.e.,* a cool temperature), and was not, as reported by earlier workers, associated with reproductive maturity.

INTRODUCTION

AMONG TERRESTRIAL gastropods, bioluminescence is known only for the Asian snail *Dyakia striata* (Godwin-Austen, 1891) (formerly in *Quantula*) (HANEDA, 1946). Light is produced by a discrete photogenic organ located near the undersurface of the foot and behind the mouth. It is yellowish green and appears as simple or modulated flashes that usually last a second or two but may last as long as 6 sec. Flashes may be repeated up to 30 times/min during peak activity just after dark (PARMENTIER & BARNES, 1975). They are mostly given by snails of 5 to 15 mm in shell width (HANEDA, 1963), although the maximum size of adults is at least 27 mm. Nearly all flashing is performed by feeding or moving snails while fully extended. The intensity of light is very low: even in complete darkness only the brightest flashes can be seen by human eyes from more than several meters or from behind the shell.

Light is reportedly also produced by luminous cells scattered over the foot and mantle. It differs from light produced by the photogenic organ in being extremely faint and continuous (HANEDA & TSUJI, 1969). It is recorded as being found in all newly hatched snails but in only 20–30% of adults (HANEDA, 1981). PARMENTIER & BARNES (1975) did not mention this type of luminescence.

Little is known about the function or functions of flashing by *Dyakia striata.* MARTOJA & BASSOT (1970) reported that the photogenic organ is replaced by a non-functional "reabsorption cyst" before the gonads mature. HANEDA (1963) had earlier observed that some snails did not luminesce, but he and TSUJI (1969) considered this phenomenon to be an individual characteristic of adults. The recent finding that some very large snails may possess both a functional photogenic organ and well-developed reproductive systems (COPELAND & MANERI, in press) further suggests that light production is not tied to reproduction. Nor does it appear to serve as a warning of the performer's unpalatability (CHAN, 1984), as does the glow of firefly larvae (CARLSON & COPELAND, 1978). Local illumination is unlikely because the light is not directional and flashes are not given when the foot is raised or when an obstacle is encountered (PARMENTIER & BARNES, 1975). Similarly, most other functions proposed for other luminescent organisms (see BUCK, 1978) are apparently not applicable to *D. striata.* For example, jamming, repelling,

and concealment are difficult to imagine as roles for flashing because the light is so weak.

Several workers have tried to evoke luminescence in *Dyakia striata* by artificial means. HANEDA (1981) subjected snails to electrical and mechanical stimulation, and to injury. As well as these methods, PARMENTIER & BARNES (1975) tried food, pure oxygen, and several neurotransmitters. All these efforts failed. COPELAND & MANERI (in press), on the other hand, reported being able to stimulate approaching and flashing responses with artificial flashes. On the basis of this evidence, these authors hypothesized that luminescence in *D. striata* facilitates aggregating behavior. However, the observed instances of approaching and flashing were few; and, the basic social and environmental conditions that may influence flashing, and could thereby promote or inhibit communication (or some other activity), were not investigated.

In this study we examine the effects of a variety of conditions on the rate of flashing by the photogenic organ and its loss of function in some snails. Two factors, age and group size, were chosen to test the hypothesized relationships of flashing with maturity (HANEDA, 1963) and with aggregating (COPELAND & MANERI, in press). Four others were selected as likely influences on the behavior of *Dyakia striata*: they were diet, starvation, photoperiod (see HODASI, 1982), and temperature (see CAMERON, 1970). An experiment was also conducted to test further the hypothesized effects of reproduction on loss of luminescence (MARTOJA & BASSOT, 1970).

MATERIALS AND METHODS

Table 1 identifies the 16 experiments that were conducted. The snails used here were collected during the period of 21 May to 11 July 1984 from several localities in the Republic of Singapore. Most came from one small area of a northeast-facing slope near a large drainage canal in Clementi New Town. An additional 798 animals were collected solely for an examination of the photogenic organ. They were obtained during the period of 20 May to 7 October 1985 from the old campus of the National University of Singapore in Bukit Timah.

Before the experiments were begun (and fortnightly thereafter), the experimental animals were examined for luminescence—whether or not the organ is flashing at the moment, it nevertheless fluoresces under UV irradiation. They were also measured for shell width. The size measurement was presumed to indicate age, because shell growth in captive snails was constant with time (LOH, 1984). Snails used in the experiments on temperature, continuous light, and continuous dark were housed in specially prepared cabinets. In the first two of these treatments, lighting was provided by single 40-W incandescent bulbs. Snails in the remaining experiments (except that on reproductive maturity) were housed in a small light-sealed room with controlled photoperiod and constant temperature. Lighting here consisted of two 40-W incandescent bulbs. In the study of reproductive maturity, snails were kept under natural shaded light conditions (of about 12 h of light per day) and natural temperatures (of 24–30°C).

The first 15 experiments were run for 13 weeks, from 1 August to 31 October 1984. The last experiment was run for 12 weeks, from 17 July to 11 October 1984.

Effects of Non-social Factors on Rate of Flashing and Loss of Luminescence (Experiments 1–8)

Because *Dyakia striata* is found in the field as lone animals and in small groups, these experiments were conducted on both isolated and grouped snails. For each experiment, 20 snails were kept separately in round plastic containers of 6.5 (height) × 9 cm (diameter); and 20 were kept in two groups of 10 in rectangular plastic containers of 16 (height) × 12 × 18 cm. All grouped snails were color-coded with nail polish. Every other day the containers were cleaned, fresh food was provided, and the wet paper towels used to keep the animals moist were replaced. Mean shell widths for these eight treatments varied from 16.6 to 17.8 mm, with individual snails ranging from 11.7 to 20.9 mm.

Because pulmonates in general survive and reproduce best on a mixed diet (RUNHAM, 1975), the control diet consisted of several foods. It was composed of frequently replenished pieces of carrot, cucumber, lettuce, and rat-chow, and an occasional piece of *Achatina fulica* (Bowdich, 1822), a large land snail on which *Dyakia striata* scavenges in the field. Table 2 gives a partial nutritional analysis of these foods.

Flashing was observed by a dark-adapted person, with the aid of a dim UV light (NIS FL4 BLB) to enhance the brightness of flashes. Whether simple or modulated, flashes were distinct from one another, and each was counted as a single flash. Data on flashing were collected every third day just after the start of the daily dark cycle (at 0900 h). Among active, non-feeding snails in each experiment, five were chosen at random from isolates and five from groups, for observations of 1 min each. Non-luminescent snails were, of course, not included; and neither were snails fed the *Achatina fulica* diet because all died within 10 days. Only a small number of observations were made on starved snails during the two weeks when most were still alive.

Effects of Group Size and Age on Rate of Flashing (Experiments 9–15)

These experiments differed from no. 1 to 8 in that isolates were not used, and dead snails or those that had become non-luminescent were replaced with luminescent snails of similar size. Maintenance conditions were the same as those for grouped snails in the control experiment. Data on flashing were collected in a similar manner as experiments 1–8 but on every second day.

Table 1

Conditions for experiments on *Dyakia striata.*

Experiment	Presence of luminescence	Diet	Photoperiod (L:D)	Temp. (°C)	Sample size*
1. Control conditions	initially	control	12:12	30	40
2. Non-luminescence	no	control	12:12	30	40
3. Cucumber diet	initially	cucumber	12:12	30	40
4. *Achatina* diet	initially	*Achatina*	12:12	30	40
5. Cool temperature	initially	control	12:12	20	40
6. Continuous light	initially	control	24:0	30	40
7. Continuous dark	initially	control	0:24	30	40
8. Starvation	initially	control	12:12	30	40
9. Groups of 2	throughout	control	12:12	30	4
10. Groups of 5	throughout	control	12:12	30	10
11. Groups of 10	throughout	control	12:12	30	20
12. Groups of 15	throughout	control	12:12	30	30
13. Young snails	throughout	control	12:12	30	20
14. Middle-age snails	throughout	control	12:12	30	20
15. Old snails	throughout	control	12:12	30	20
16. Reproductive maturity	initially	control	natural	natural	120
				Total:	564

* For experiments 1–8, these are initial sample sizes, because dead snails were not replaced.

Mean shell widths for treatments 9–12 varied from 16.1 to 17.4 mm, with individual snails ranging from 13.5 to 21.3 mm. For experiments 13–15, young snails averaged 13.6 mm (10.0–14.6), middle-age snails 17.0 mm (15.8–17.7) and old snails 20.0 mm (18.8–21.1).

Effects of Reproductive Maturity on Loss of Luminescence (Experiment 16)

Forty isolated snails and 40 pairs, all initially luminescent, were kept in small round containers (see above) with soil for egg-laying. The soil was examined for eggs every other day and changed if fouled. All snails were fed the control diet. Their mean shell width was 18.8 mm (11.9–25.5). Unfortunately, too few animals were available to run a parallel experiment on breeding by non-luminescent

Table 2

Nutritional analysis of diets fed *Dyakia striata,* in percent.

Constituent	Food				
	Cucumber	Carrot	Lettuce	*Achatina*	Rat-chow
Moisture	96.9	92.5	95.6	84.3	12.5
Dry matter	3.1	7.5	4.4	15.7	87.5
	100.0%	100.0%	100.0%	100.0%	100.0%
Crude protein	18.8	6.7	24.4	60.8	24.0
Crude fat	6.1	5.1	4.6	6.6	3.0
Ash	15.2	13.5	15.8	12.4	8.5
Nitrogen-free extract*	59.9	74.7	55.2	20.2	64.5
	100.0%	100.0%	100.0%	100.0%	100.0%

* Primarily carbohydrates.

Table 3

Flashing rates per minute by *Dyakia striata.**

Experiment	Number of minutes (*n*)	Mean	SE	Minimum	Maximum
Isolates					
1. Control conditions	165	24.8	0.34	16	38
3. Cucumber diet	165	27.6	0.36	18	38
5. Cool temperature	135	13.6	0.33	6	19
6. Continuous light	208	22.4	0.28	16	35
7. Continuous dark	220	22.7	0.28	15	33
Groups					
1. Control conditions	165	26.7	0.38	18	42
3. Cucumber diet	165	30.1	0.38	19	48
5. Cool temperature	165	13.6	0.29	7	25
6. Continuous light	213	22.2	0.30	13	34
7. Continuous dark	220	24.1	0.30	17	35
9. Groups of 2	184	22.0	0.31	14	34
10. Groups of 5	480	25.5	0.23	16	42
11. Groups of 10	960	23.1	0.14	13	39
12. Groups of 15	1440	20.6	0.11	12	38
13. Young snails	960	27.4	0.14	17	39
14. Middle-age snails	960	23.2	0.14	14	36
15. Old snails	960	20.8	0.15	13	37

* Excludes experiments on *Achatina fulica* diet (no. 4) and starvation (no. 8), because all snails soon died, and experiment on non-luminescence (no. 2).

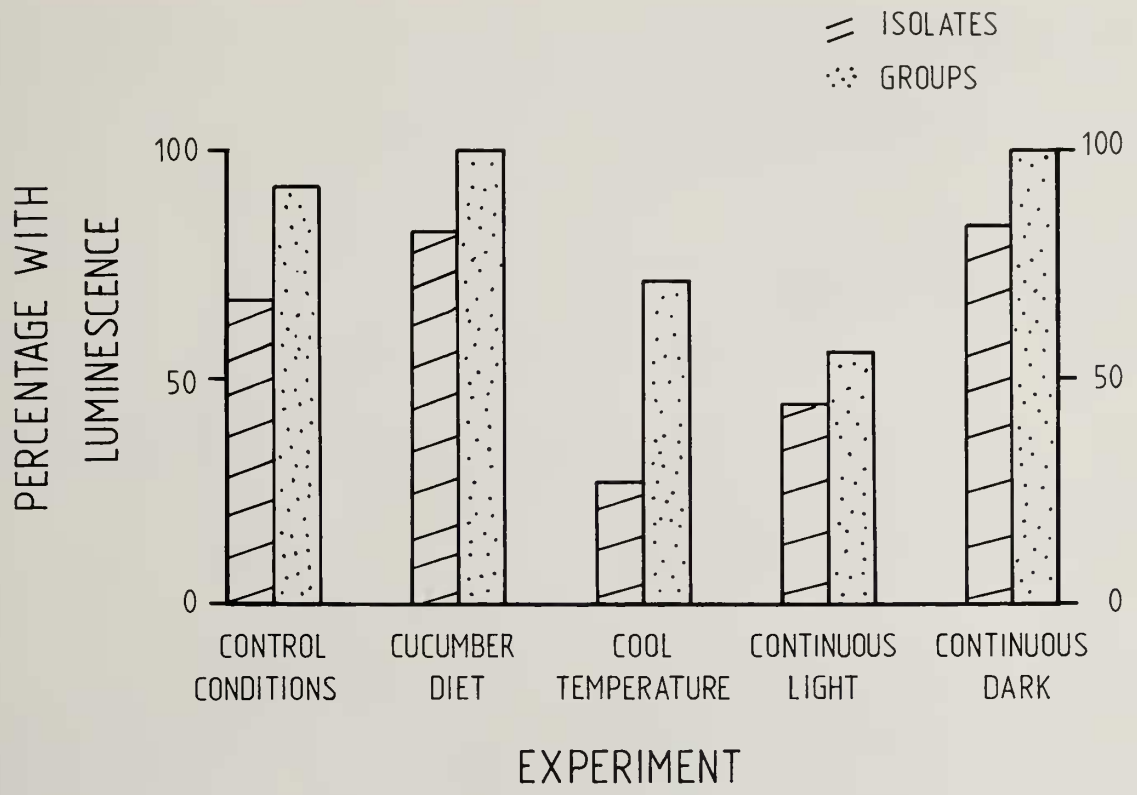

Figure 1

Percentages of luminescent snails, *Dyakia striata*, among 117 snails surviving until the end of the study.

individuals. *Dyakia striata* is a simultaneous hermaphrodite.

RESULTS

Mortality

Numbers of dead snails were recorded for the first eight experiments. All snails fed the *Achatina fulica* diet died within four weeks; and most of those not fed at all died within five weeks, although one snail survived seven weeks. Among the remaining experiments, there were no significant differences, whether isolates and groups were combined in a one-sample test ($\chi^2 = 6.0$; d.f. = 5; $P > 0.05$) or separated into a two-sample test ($\chi^2 = 1.4$; d.f. = 5; $P > 0.05$). Thus luminescent and non-luminescent snails had similar mortality rates.

Flashing Rates

Among the non-social treatments for which data were in adequate numbers (*i.e.*, control conditions, cucumber diet, cool temperature, continuous light, and continuous dark), the number of flashes per minute varied considerably, although not in the same manner for both group sizes (*i.e.*, isolates and groups of 10). This finding was revealed by a highly significant interaction between experimental type and group size ($F = 6.3$; d.f. = 4, 1811; $P < 0.001$). A further analysis, using a Student-Newman-Keuls multiple range test, was conducted on the 10 combinations of group size and experimental type. The test identified five significantly separate subsets: (1) groups on cucumber diet, (2) isolates on cucumber diet and groups under control conditions, (3) isolates under control conditions and groups in continuous dark, (4) isolates in continuous dark, and isolates and groups in continuous light, and (5) isolates and groups under the cool temperature. Table 3 gives the basic statistics for these classes and shows that the first subset had the highest flashing rate and the last subset had the lowest.

Although the sample of flashing from starved snails was, we judged, too small for detailed comparisons with other experiments, the mean number of flashes per minute for both isolates and groups combined ($\bar{X} = 14.8$; SE = 0.37; $n = 80$ min) indicated that these snails flashed comparatively little. Their rate was only slightly higher than the combined rate for isolated and grouped snails under the cool temperature ($\bar{X} = 13.6$; SE = 0.31; $n = 300$ min).

Snails in groups of five had the highest rate and those in groups of 15 had the lowest (Table 3). A one-way analysis of variance on the number of flashes per minute

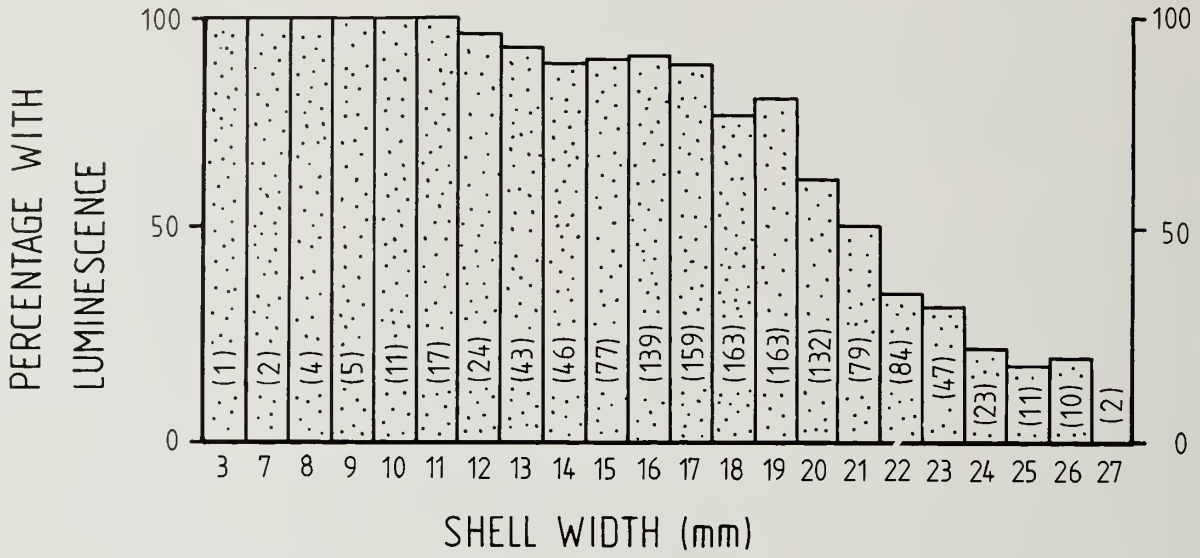

Figure 2

Percentages by shell width of luminescent snails, *Dyakia striata,* among 1242 newly captured snails (sample size per mm in parenthesis).

by snails in groups of different sizes was highly significant ($F = 170.4$; d.f. = 3, 3060; $P < 0.0001$), and a Student-Newman-Keuls test revealed that every group size differed significantly from every other size.

For the experiments on age, young snails flashed most and old snails flashed least (Table 3). A one-way analysis of variance on number of flashes per minute was highly significant ($F = 564.1$; d.f. = 2, 2877; $P < 0.0001$), and the Student-Newman-Keuls test revealed significant separation of all three age classes.

Loss of Luminescence

Excluding the experiment on non-luminescent snails, five treatments among the first eight had snails surviving until the end of the study (Figure 1). Neither the variations among experiments (Friedman test: $\chi^2_r = 7.3$; d.f. = 4; $P > 0.05$) nor those between the two group sizes (sign test: $x = 0$; $n = 5$; $P > 0.05$) were significant. However, in the latter test isolates and groups were nearly significantly different ($P = 0.06$), and within every experiment isolates accounted for a larger proportion of non-luminescent animals than did groups. Moreover, among snails fed the *Achatina fulica* diet and those starved, only isolates (eight and four respectively) had become non-luminescent before dying.

No snail within the first eight experiments regained the ability to luminesce after having lost it. This finding is supported by data on snails examined immediately after capture, which showed that the proportion of luminescent animals decreased with increasing shell size (Figure 2). In total, 73.4% of the sample was luminescent just after capture.

In the experiment to test the effects of reproductive maturity (no. 16 in Table 1), six isolates (ranging in size from 17.8 to 21.5 mm) laid three clutches of over 20 eggs each and four clutches of 1–4 eggs each. The large clutches were laid by snails that were luminescent both before and after laying. All three produced some live young. Three of the small clutches were also laid by luminescent snails that retained their ability to luminesce after laying, but one was produced by a non-luminescent animal. None of these clutches was viable.

Among the 40 pairs in the experiment on reproduction, only one snail (19.6 mm) became non-luminescent; neither it nor its companion laid eggs. Eighteen of the other pairs laid 34 clutches of 4–38 eggs, and one pair laid single eggs on two occasions. Except for three clutches eaten by adults and the single eggs, all clutches produced live young. Five of the 18 pairs laid three or four clutches each, which revealed that even multiple laying by an individual snail did not result in loss of luminescence. Members of pairs that laid viable eggs ranged in size from 16.5 to 25.5 mm.

DISCUSSION

Our results strengthen four previous, unsubstantiated conclusions regarding luminescence in *Dyakia striata,* namely, that (1) it is present in all young snails but in decreasing proportions of snails with increasing age (Haneda & Tsuji, 1969), (2) its loss, once having occurred, is permanent (Martoja & Bassot, 1970), (3) it is not influenced by reproductive development (Copeland & Maneri, in press), and (4) it most likely serves an intraspecific social function (Copeland & Maneri, in press). The experiments also revealed a considerable sensitivity by *D. striata* in its flashing behavior to a wide

range of social and environmental conditions. Some of the results for loss of luminescence were less clear; but they appeared to indicate that functioning of the photogenic organ is affected by some adverse conditions (*i.e.*, isolation, and perhaps also cool temperatures and excessive light) but not others (*i.e.*, starvation and diet).

As mentioned in the Introduction, MARTOJA & BASSOT's (1970) hypothesis that *Dyakia striata* becomes non-luminescent when the gonads mature seemed unlikely on recent evidence. The finding that luminescent snails can repeatedly lay clutches of viable eggs disproves it—indeed, whether non-luminescent snails can reproduce successfully remains to be seen. Rather, our results support HANEDA & TSUJI's (1969) unquantified assertion that loss of luminescence is an individual characteristic of adult snails. For example, within the collection of freshly captured snails, one snail had become non-luminescent by 12 mm, while two were still capable of producing light at 26 mm, nearly the maximum size for this species (see Figure 2). Both luminescent and non-luminescent forms of *D. striata* had identical growth rates during the three months of study (LOH, 1984), as well as similar mortality rates.

HANEDA (1963, 1979) reported that snails from 5 to 15 mm flash most. Our experiments on body size confirmed that flashing declines with age, at least in snails of 10 mm and over. In addition, the few snails under 10 mm that we observed had brighter flashes than those of reproductive size, and many luminescent animals over 20 mm had very dim lights.

A social role for luminescence in *Dyakia striata* is supported by results from our experiments on control conditions, cucumber diet, and continuous dark, in which grouped snails flashed more than did isolated snails (see Table 3). In addition, fewer grouped than isolated snails in every one of the first eight experiments (except, of course, that on non-luminescent snails) lost the ability to flash (see Figure 1 and text). Some of the experiments may even be interpreted as providing evidence for an ecological link between flashing and grouping behaviors. In the experiment on group size, groups of five flashed most; and five is a common, if not the most common, number of snails found together in the field on rotting meat, fruit, or vegetables. Among the food-related treatments (including starvation), rates of flashing varied considerably (see Table 3 and text), but losses of luminescence did not (see Figure 1 and text). Lastly, among all experiments rates were highest for the cucumber diet.

Taken together, our findings suggest that flashing in *Dyakia striata* promotes the aggregation of young snails on sources of food that are probably widely scattered in space or time, or both. We stress, however, that the experiments were not designed to give direct evidence of light communication. Moreover, any hypothesis on communication among non-reproductive *D. striata* faces a major theoretical problem: according to present theory (*e.g.*, TRIVERS, 1985), it requires kin selection, or the unlikely alternative explanation that flashing snails are behaving altruistically towards other snails that may or may not be relatives. There is currently no information on kin-selected behavior in *D. striata*. The few data available on the reproductive biology and ecology of this species (*e.g.*, large clutches and restricted microhabitats) do not preclude the possibility.

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